Transactions of the Illinois State Academy of Science
(1983), Volume 76, 3 and 4, pp. 265-269

Lignin Analyses on Sweet Sorghum Samples

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ABSTRACT

A comparison of lignin contents determined by two methods on two replicated samples receiving one of three pretreatments is reported for freeze-dried sweet sorghum. The pretreatments were: extraction with 95% ethanol/benzene (1:2, v/v); extraction with 80% aqueous ethanol; and no extraction prior to lignin analysis by ultraviolet spectrophotometric (UV) or sulfuric acid methods. Multiway analysis of variance was used to examine results, and highly significant variation among treatments and methods was observed. Differences in lignin content between samples and between methods were greatest when the sweet sorghum was not extracted prior to analysis. These differences were reduced when samples were first extracted with alcohol/benzene. Differences between samples and between methods virtually vanished when an 80% aqueous ethanol extraction preceded lignin analysis. The results illustrate the limitations of these lignin analysis methods when applied to a material like sweet sorghum. A pretreatment consisting of 80% aqueous ethanol extraction appears to provide a suitable substrate for either method of lignin analysis on sweet sorghum.

INTRODUCTION

We recently reported lignin contents of sweet sorghum [*Sorghum bicolor* (Moench), variety Wray] as part of a compositional study on freeze-dried whole stalk and bagasse samples harvested from sewage sludge-amended stripmine soils (Carlson *et al.*, 1983). The method used was that of Bagby *et al.* (1973), in which lignin is solubilized in acetyl bromide and determined by ultraviolet (UV) absorption at 280 nm. In developing this method, these workers showed that the differences in lignin content determined for samples of green kenaf (*Hibiscus cannabinus L.*) both before and after 95% ethanol/benzene (1:2, v/v) extraction was not signifi-

[1]The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

cant, but that the precision without the extraction was lower when all data from the study were included (green and post-frost kenaf, and kenaf pulps). The UV method was highly correlated ($r = 0.97$) with a previously developed sulfuric acid gravimetric method (Bagby *et al.*, 1971), but lignin contents determined by the two methods for kenaf pulps were significantly different ($P < 0.05$). Generally, either method has proved satisfactory for determining lignin in nonwood, low-extractive, agricultural-type materials when preceded by the alcohol/benzene extraction step. In this note, comparison of the two methods as applied to sweet sorghum samples shows that results differ significantly depending upon pretreatment of the sweet sorghum samples, and a pretreatment is suggested that gives consistent results by either method of lignin analysis.

MATERIALS AND METHODS

One sample of sweet sorghum (60C, Table 1) was grown in central Illinois as previously described (Carlson, *et al.*, 1983). The second sample (25A, Table 1) was a composite of sweet sorghum grown in five locations in Michigan and received frozen. Fresh (IL) or frozen (MI) stalks cut into short segments were split longitudinally, freeze-dried, and finally ground to pass a 1-mm screen. Each sample contained approximately 50% total sugars (dry basis). Alcohol (95%)/benzene (1:2, v/v) or 80% aqueous ethanol (ethanol/water, 80:20, v/v) extractions were made in a Soxhlet extractor for 18 hr on 10-g samples in duplicate. Solubles removed from sample 60C were 25.1% (alcohol/benzene) and 61.5% (80% aqueous ethanol); from sample 25A, solubles were 51.9% (alcohol/benzene) and 55.7% (80% aqueous ethanol) of the sample dry weight. Lignin analyses were made by the sulfuric acid (Bagby *et al.*, 1971) and UV spectrophotometric methods (Bagby *et al.*, 1973). Data were examined by analysis of variance based on two sorghum samples, two methods, three extraction pretreatments, and two replicates of each combination. The least significant ratio for comparing two means was determined (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Wall and Blessin (1970) tabulated compositional data for different sorghums that had lignin contents ranging from 10% to 17%, the lower values for sweet sorghums and the higher values for grain and grass sorghums. The UV method for lignin determination (Bagby *et al.*, 1973), as applied to our sweet sorghum, similarly gave lignin contents of 12 to 20% for alcohol/benzene extracted, freeze-dried stalks (Carlson *et al.*, 1983). Alcohol/benzene extraction removed an average of 25% of the dry weight of these samples, the same as previously reported for sweet sorghum extractives (Wall and Blessin, 1970).

We became increasingly aware of interfering absorption in UV lignin determinationss on many sorghum samples that are routinely received and screened for sugars and other constituents. We presumed that the problem was associated with the presence of high levels of extractives in many of these samples, and an alternate extraction with 80% aqueous ethanol was devised to remove both sugars and interfering UV absorbers (possibly tannins, etc.). This technique provided substrates with much lower apparent lignin than either unextracted or alcohol/benzene extracted sorghum. We tested the effectiveness of the pretreatments by comparing

the UV lignin analysis against the gravimetric sulfuric acid method for lignin. Ideally, the two methods will agree when interfering UV absorbers have been removed, assuming, of course, that the sulfuric acid method does not destroy lignin and that the extraction solvents neither destroy nor remove lignin from the sample.

The results of our study are shown in Table 1. Mean lignin contents are listed for two samples that received no preparatory treatment or one of two extraction pretreatments prior to lignin analysis. Highly significant variation among treatments was observed. The least significant ratio (LSR) of two means (sample, treatment, or method) is 1.086; two means with different superscripts are significantly different ($P < 0.05$). The overall relative standard deviation among sample replicates was 3.9%, indicating that determinations by either method for a particular sample were highly reproducible. The three- to sixfold range in UV lignin values, depending upon sample and pretreatment, illustrates the unreliability of the method for a high-extractives material like sweet sorghum unless interfering substances are removed with an appropriate solvent. Agreement among the sulfuric acid lignin data, regardless of sample and pretreatment, is substantially better but differences are still significant, suggesting that this method is also sensitive to substances associated with the extractives. Differences in lignin content, by either method, are significant for samples receiving the same pretreatment when the pretreatment is either no extraction or alcohol/benzene extraction. However, when the extractant is 80% aqueous ethanol, apparent lignin contents converge near 5%, and only the UV value for sample 25A is statistically different from the other three values.

This convergence is shown in Figure 1, where apparent lignin contents (%) for the two samples in Table 1 and additional UV lignin data for four other samples are plotted as a function of extractives (%) removed by each extractant. Linear regression analysis on the data gave the equations:

$$\text{Lignin (UV)} = 24.63 - 0.332\ \text{Extractives} \qquad (r = -0.95)$$

$$\text{Lignin (Sulfuric acid)} = 7.29 - 0.0373\ \text{Extractives} \qquad (r = -0.75)$$

UV lignin is highly correlated with extractives. The slope of the sulfuric acid lignin line was not significantly different from zero, but more data are needed to test further the sensitivity of the sulfuric acid method to interference by extractives of sweet sorghum. The sulfuric acid method gives a reasonable estimate of lignin in unextracted sweet sorghum. Convergence of the two methods occurs near 59% extractives with an apparent lignin content near 5.1%.

We found that a second extraction with alcohol/benzene was as effective as a single 80% aqueous ethanol extraction in bringing the apparent UV lignin into line with the sulfuric acid method and the 80% aqueous ethanol value. For example, on the same sorghum sample, UV lignin of 10.4% and 35.9% extractives were determined in the single alcohol/benzene extraction; after the second alcohol/benzene extraction the values were 5.4% lignin and 56% extractives, compared with 5.5% UV lignin and 59.2% extractives in the single 80% aqueous ethanol extraction.

It is conceivable that 80% ethanol extraction or treatment with sulfuric acid removes or destroys lignin, which would result in low lignin values by either method. It is also possible that agreement between methods is fortuitous when the extractant is 80% aqueous ethanol. Results given in the previous paragraph argue against either of these possibilities. From our results it is apparent that 80%

aqueous ethanol extraction of sweet sorghum provides a suitable substrate for obtaining comparable apparent lignin contents either by the faster UV method or by the more consistent sulfuric acid method. Exhaustive alcohol/benzene extraction is also effective in preparing sweet sorghum samples for UV lignin analysis.

ACKNOWLEDGMENTS

We thank Dr. William Kwolek for advice and assistance with the statistical analyses.

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Table 1. Apparent lignin contents of freeze-dried sweet sorghum stalks as determined by two methods on samples receiving different solvent extractions prior to analysis.

	Lignin, % (Dry Basis)*			
	UV Method#		Sulfuric Acid Method+	
Extractant‡	Sample 60C	Sample 25A	Sample 60C	Sample 25A
None	30.36[a]	19.80[b]	5.78[f]	8.52[d]
95% Ethanol/benzene (1:2, v/v)	16.68[c]	7.32[e]	6.92[e]	5.12[g]
80% Aqueous ethanol (80:20, v/v)	5.27[g]	6.16[f]	5.09[g]	5.08[g]

*Means of duplicate analyses; means with different superscripts are significantly different ($P < 0.05$). Least significant ration (LSR) of means = 1.086. Relative standard deviation (RSD) = 3.9%.
#Method of Bagby *et al.* (1973), by ultraviolet (UV) absorption at 280 nm.
+Method of Bagby *et al.* (1971).
‡Soxhlet extractions (18 hr), prior to analysis.

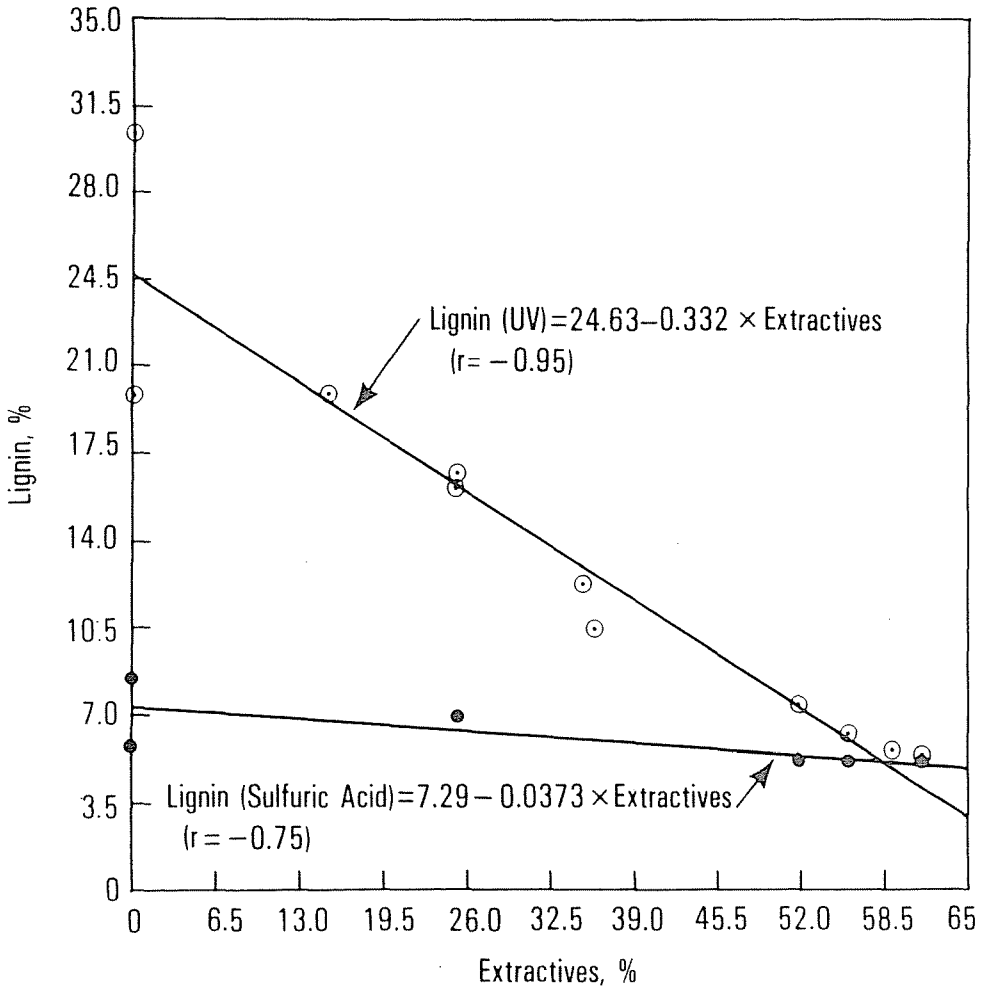

Fig. 1. Apparent lignin contents of sweet sorghum samples as a function of extractives (% of sample dry weight solubilized during alcohol/benzene or 80% aqueous ethanol extraction).